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Short communication

Microbiological assay for gatifloxacin in pharmaceutical formulations

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Abstract

A simple, sensitive and specific agar diffusion bioassay for the antibacterial gatifloxacin was developed using a strain of *Bacillus subtilis* ATCC 9372 as the test organism. Gatifloxacin could be measured in tablets and raw material at concentration ranging 4–16 μg ml^{-1}. The calibration graph for gatifloxacin was linear from 4.0 to 16.0 μg ml^{-1}. A prospective validation of the method demonstrated that the method was linear (r^2 = 0.9993), precise (R.S.D. = 1.14%) and accurate. The results confirmed its precision and did not differ significantly from others methods described in the literature. The validated method yielded good results in terms of the range, linearity, precision, accuracy, specificity and recovery. We concluded that the microbiological assay is satisfactory for in vitro quantification of the antibacterial activity of gatifloxacin.

Keywords: Gatifloxacin; Fluoroquinolones; Microbiological assay; Pharmaceutical analysis; Quality control; Cylinder plate method

1. Introduction

Gatifloxacin (GATX) is a new 6-fluoro-8-methoxy quinolone with a wide spectrum of activity against aerobic and anaerobic bacteria, including antibiotic-resistant *Streptococcus pneumoniae* [1]. It has been suggested that gatifloxacin (Fig. 1) possess potent antipneumococcal activities and select mutant strains less frequently than other fluoroquinolones because of their inhibition of DNA gyrase [2]. Gatifloxacin has a characteristic methoxy group at the 8-position of the quinolone ring.

The contributions of the methoxy groups of certain fluoroquinolones, including gatifloxacin, to antibacterial activity and/or resistance selectivity have been investigated in some bacteria. The methoxy group has been shown to correlate with the prevention of emergence of the mutant strains and/or potent in vitro activity against *Escherichia coli* [3], *S. aureus* [4] and mycobacteria [5]. This fluoroquinolone has high oral bioavailability (96%), and therefore, oral and intravenous formulations are bioequivalent and interchangeable [6]. Chemically gatifloxacin is a (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate and is not yet official in any pharmacopoeia [7,8].

The literature has reported microbiological assays for determination of fluoroquinolones in pharmaceutical formulations, such as ofloxacin [9] and sparfloxacin [10]. However, the microbiological assay for the determination of GATX in raw material and tablets has not yet been reported. This assay can reveal subtle changes not demonstrable by chemical methods. Moreover, it gives the possibility to evaluate the potency of GATX, which is very important for the analysis of antibiotics. The aim of this study was to validate an agar diffusion method through the parameters linearity, precision and accuracy to quantify GATX in raw material and tablets.

2. Materials and methods

The gatifloxacin reference substance (assigned purity 99.99%) as well as gatifloxacin tablets were generous gifts from Bristol–Myers Squibb (Brazil). The tablets were claimed to contain 400 mg of drug and excipients. The GATX reference substance, as well as the tablets, was always kept

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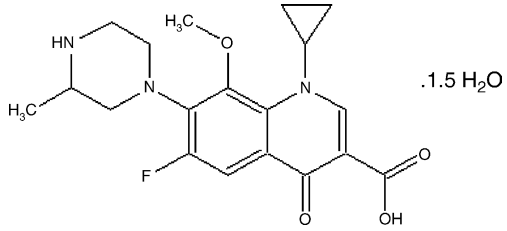

Fig. 1. Chemical structure of gatifloxacin (CAS number 160738-57-8).

protected from light. All chemicals and reagents were of analytical grade. Water was always doubly distilled.

2.1. Preparation of gatifloxacin reference substance

The standard solution in water (100 μg ml^{-1}) was diluted in potassium phosphate buffer pH 6.0 and assayed at concentrations of 4.0, 8.0 and 16.0 μg ml^{-1}.

2.2. Preparation of gatifloxacin in tablets

Twenty tablets were weighed and pulverized. An amount of powder equivalent to 10 mg of gatifloxacin was transferred to a 100 ml volumetric flask with 50 ml water and shaken for 10 min. This was followed by making up to volume with water. The dilutions were made with potassium phosphate buffer pH 6.0 to give a final concentration of 4.0, 8.0 and 16.0 μg ml^{-1}.

2.3. Organism and inoculum

The cultures of *Bacillus subtilis* ATCC 9372 were cultivated on Grove Randall number 1 agar (Merck) in the freezer and pealed to another grove Randall number 1 agar (24 h before the assay) that was kept at 37 °C. The bacteria were suspended in tryptic soy broth (Difco) using a glass homogenizer. Diluted cultures suspension of 25 ± 2% turbidity were obtained at 580 nm, using a suitable spectrophotometer and a 10 mm diameter test tube as an absorption cells against tryptic soy broth as blank. Portions of 1.0 ml of the inoculated tryptic soy broth were added to 50 ml of Grove Randall number 11 agar (Merck) at 47 ± 2 °C and used as the inoculated layer.

2.4. Cylinder plate assay

The agar was composed of two separate layers. The Grove Randall number11 agar (20 ml) was poured into 100 mm × 20 mm Petri dish as a base layer. After solidification portions of 5 ml of inoculated Grove Randall number 11 agar were poured onto the base layer. Six stainless steel cylinders of uniform size (8 mm × 6 mm × 10 mm) were placed on the surface of the inoculated medium. Three alternated cylinders were filled with 200 μl of the reference solutions and the other three were filled with the sample solutions. After incubation (37 °C for 18 h), the zone diameters (in mm) of the growth inhibition were measured using a caliper (Starret).

2.5. Calculation

To calculate the activity of gatifloxacin in tablets the Hewitt [11] equation was used. The assay was statistically analysed by the linear parallel model and by means of regression analysis of variance [8,11].

2.6. Method validation

The method was validated by determination of linearity, precision and accuracy [8,12].

- *Linearity*: In order to assess the validity of the assay three doses of the reference substance and three doses of the sample were used. The calculation of regression line by the method of least squares was employed.
- *Precision*: Reproducability (intra-assay) and intermediate precision (inter-assay) were determined. Method repeatability was studied by analyzing samples of tablets, at the same concentration, within one day and under the same experimental conditions. The intermediate precision was evaluated by comparing the assays on different days.
- *Accuracy*: Accuracy was determined by adding known amounts of GATX reference substance to the samples at the beginning of the process. Accurately weighted amounts of tablets equivalent of 10 mg GATX were placed in three 100 ml volumetric flask. 0.5, 1.0 and 1.5 ml of gatifloxacin reference solution (100 μg ml^{-1}) were added. Water (50 ml) was added and the flasks were shaken for 10 min. This was followed by making up to volume with water. The dilutions were made in potassium phosphate buffer, pH 6.0, to give a final concentrations of 105.0, 110.0 and 115.0%, respectively. The solutions were applied the cylinder plate assay described above. The percentage recovery of GATX reference added was calculated using the formula proposed by the AOAC [13].

3. Results and discussion

In this work, an experimental 3 × 3 design, using three dose levels for each standard and sample, was used following the procedure described in the Brazilian [14] and European Pharmacopoeias [15].

The calculation procedure usually assumes a direct relationship between the observed zone diameter and the logarithm of applied dose. The corresponding mean zone diameters for reference solutions were: 19.71 ± 0.19 mm (R.S.D. = 1.0) for low dose, 21.51 ± 0.13 mm (R.S.D. = 0.6) for medium dose and 23.15 ± 0.05 mm (R.S.D. = 0.22) for high dose. Corresponding zone diameters ranged between 19.49 and 19.86, 21.36 and 21.60, and 23.10 and 23.20 mm for concentrations of 4.0, 8.0 and 16.0 μg ml^{-1}, respec-

Table 1
Results of diameter zone of inhibition for gatifloxacin reference solutions

Concentration ($\mu g\ ml^{-1}$)	Mean diameter zone of inhibition (mm) ± R.S.D.	Range of zone size (mm)	CV%
4.0	19.71 ± 0.19	19.49–19.86	1.00
8.0	21.51 ± 0.13	21.36–21.60	0.61
16.0	23.15 ± 0.05	23.10–23.20	0.22

CV = coefficient of variation; R.S.D. = relative standard deviation; $n = 6$.

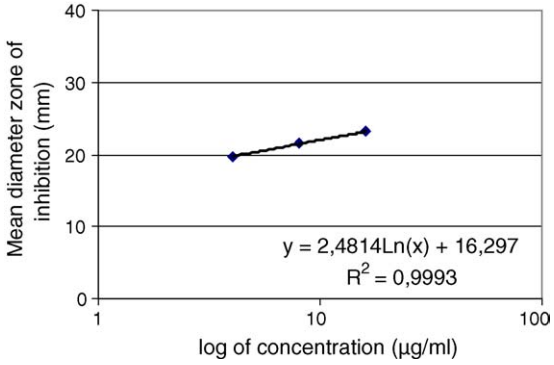

Fig. 2. Log of concentrations ($\mu g\ ml^{-1}$) vs. mean diameter zone (mm) from the assay of GATX by 3 × 3 design.

tively (Table 1). The calibration curves for GATX were constructed by plotting log concentrations ($\mu g\ ml^{-1}$) versus zone diameter (mm) and showed good linearity between 4.0 and 16.0 $\mu g\ ml^{-1}$ range (Fig. 2). The representative linear equation for GATX was $y = 5.71 \ln x + 16.29$, where x is log dose and y is zone diameter. The coefficient of regression was $r^2 = 0.9992$.

The experimental values obtained for the determination of GATX in the samples are presented in Table 2. According to the British, European and Brazilian Pharmacopoeias, if a parallel-line model is chosen, the two log dose–response lines of the preparations to be examined as well as the reference preparation must be parallel and they must be linear over the range of doses used in the calculation. These conditions must be verified by validity tests for a given probability, usually $P = 0.05$.

The assays were validated by means of the analysis of variance, as described in these official codes. There was no deviation from parallelism and linearity in the results obtained here ($P < 0.05$). The precision and accuracy of the assay was also demonstrated. Precision is usually expressed as the variance, relative standard deviation (R.S.D.) or coefficient of variation (CV%) of a series of measurements [12]. The results obtained on different days show a relative standard deviation of 1.14% for tablets (Table 2).

Table 2
Data obtained in the analysis of gatifloxacin in tablets using the microbiological assay

Sample[a]	Experimental amount (mg)[b]	% Level	R.S.D.%
1	396.88	99.22	
2	400.52	100.15	1.14
3	406.04	101.51	

R.S.D. = relative standard deviation; $n = 3$.
[a] Theoretical amount: 400 mg per tablet.
[b] Each value is the mean of six analysis.

Table 3
Experimental values obtained in the recovery test for GATX samples in the form of tablets

Spiked amount of reference	Recovery amount of reference	Recovery (%)
0.5	0.48	97.8
1.0	0.96	96.4
1.5	1.48	99.3

$n = 3$.

The accuracy is shown by the agreement between the accepted value and the value found to be 99.3% for tablets (Table 3). The results obtained with the cylinder plate assay were comparable with declared amounts and with those obtained by HPLC and UV spectrophotometry. Analysis of variance indicated no significant differences between these methods ($P < 0.05$).

The quantification of antibiotic components by chemical methods such as HPLC and UV spectrophotometry, although precise, cannot provide a true indication of biological activity. Attempts to correlate antibiotic bioassay results with those from chemical methods have proved disappointing. Therefore, bioassays continue to play an essential role in manufacturing and quality control of antibiotic medicines, and still demand considerable skill and expertise to assure success [16].

Although the biological assays have a high variability, the analysis of the obtained results demonstrated that the proposed method might be very useful for determination of this drug in pharmaceutical dosage forms.

4. Conclusion

The results indicated that the microbiological cylinder plate assay demonstrated good linearity, precision and accuracy at concentration ranging from 4.0 to 16.0 $\mu g\ ml^{-1}$, therefore, being an acceptable alternative method for the routine quality control of GATX in raw materials and tablets. The method uses simple reagents, with minimum sample preparation procedures, encouraging its application in routine analysis.

Acknowledgements

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